

Appendix 2
Pending Claims

- 1
90. A method, comprising:
- a) providing:
 - i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region comprising a double-stranded region, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid;
 - ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions;
 - iii) a second oligonucleotide capable of binding to a portion of said first non-contiguous single-stranded region; and
 - iii) a cleavage agent;
 - b) mixing said target nucleic acid, said bridging oligonucleotide, said second oligonucleotide, and said cleavage agent under conditions such that a cleavage structure is formed from said target nucleic acid, said bridging oligonucleotide, and said second oligonucleotide, and wherein either said second oligonucleotide or said bridging oligonucleotide is cleaved by said cleavage agent.
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91. The method of Claim 90, wherein said cleavage agent comprises a nuclease.
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92. The method of Claim 91, wherein said cleavage agent comprises a thermostable 5' nuclease.
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93. The method of Claim 92, wherein said thermostable 5' nuclease comprises a modified polymerase; wherein said modified polymerase is a modified native polymerase of *Thermus* species.

5 94. The method of Claim 91, wherein said nuclease is selected from the group consisting of *Pyrococcus woessii* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, and *Archaeoglobus fulgidus* FEN-1 endonuclease.

6 95. The method of Claim 90, wherein said conditions of said mixing allow for hybridization of said bridging oligonucleotide and said second oligonucleotide to said target nucleic acid so as to define a region of overlap of said oligonucleotides.

7 96. The method of Claim 95, wherein said region of overlap comprises one base.

8 97. The method of Claim 95, wherein said region of overlap comprises more than one base.

9 98. The method of Claim 90, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

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99. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region, said intervening region comprising a first double-stranded portion and a second double-stranded portion separated by a connecting single-stranded portion, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid; and

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions; and

b) mixing said target nucleic acid and said bridging oligonucleotide under conditions such that said bridging oligonucleotide hybridizes to said target to form an oligonucleotide/target complex.

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100. The method of Claim 99, wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

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101. A method, comprising:

a) providing:

i) target nucleic acid comprising first and second non-contiguous single-stranded regions separated by an intervening region comprising a double-stranded portion, wherein said target nucleic acid comprises at least a portion of Hepatitis C virus nucleic acid;

ii) a bridging oligonucleotide capable of binding to said first and second non-contiguous single-stranded regions; and

iii) a reactant selected from the group consisting of polymerases and ligases; and

b) mixing said target nucleic acid, said bridging oligonucleotide and said reactant under conditions such that said bridging oligonucleotide hybridizes to said target nucleic acid, and wherein said bridging oligonucleotide is modified by said reactant to produce a

modified oligonucleotide.

~~13~~ 102. The method of Claim ~~101~~¹², wherein said reactant is a polymerase, and said modified oligonucleotide comprises an extended oligonucleotide.

~~14~~ 103. The method of Claim ~~101~~¹², wherein said reactant is a ligase, and said modified oligonucleotide comprises a ligated oligonucleotide.

~~15~~ 104. The method of Claim ~~101~~¹², wherein said bridging oligonucleotide is capable of binding to fewer than ten nucleotides of each of said first and second non-contiguous single-stranded regions.

~~16~~ 105. The method of Claim ~~104~~¹⁵, wherein said bridging oligonucleotide is capable of binding to seven or fewer nucleotides of each of said first and second non-contiguous single-stranded regions.

~~17~~ 106. The method of Claim ~~101~~¹², wherein said Hepatitis C virus is selected from the group consisting of Hepatitis C virus variants 1a, 1b, 2a/c, and 3a.

~~18~~ 107. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

- a) providing:
 - i) a cleavage agent;
 - ii) Hepatitis C virus target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;
 - iii) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid;
 - iv) a second oligonucleotide comprising a 3' portion and a 5' portion,

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wherein said 5' portion is completely complementary to said second portion of said target nucleic acid;

b) mixing said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said portion of said first oligonucleotide is annealed to said first region of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

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~~108.~~ The method of Claim ¹⁸~~107~~, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.

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~~109.~~ The method of Claim ¹⁸~~107~~, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

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~~110.~~ The method of Claim ¹⁸~~107~~, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

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~~111.~~ The method of Claim ¹⁸~~107~~, wherein either said first oligonucleotide or said second oligonucleotide comprises a fluorescent label and said detecting the cleavage of said cleavage structure comprises detection of fluorescence from said fluorescent label.

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~~112.~~ The method of Claim ¹⁸~~107~~, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

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~~113.~~ The method of Claim ¹⁸~~107~~, wherein said first and second oligonucleotides collectively comprise a fluorescence energy donor and a fluorescence energy acceptor and

wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer between said fluorescence energy donor and said fluorescence energy acceptor.

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~~114.~~ The method of Claim ¹⁸~~107~~, wherein said either said first oligonucleotide or said second oligonucleotide comprises a label selected from the group consisting of a radioactive label, a luminescent label, a phosphorescent label, a fluorescence polarization label, and charge label, and detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge from said label.

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~~115.~~ The method of Claim ¹⁸~~107~~, wherein said first oligonucleotide is attached to a solid support.

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~~116.~~ The method of Claim ¹⁸~~107~~, wherein said second oligonucleotide is attached to a solid support.

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~~117.~~ The method of Claim ¹⁸~~107~~, wherein said cleavage agent comprises a structure-specific nuclease.

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~~118.~~ The method of Claim ²⁸~~117~~, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

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~~119.~~ The method of Claim ²⁹~~118~~, wherein said cleavage agent comprises a 5' nuclease.

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~~120.~~ The method of Claim ³⁰~~119~~, wherein said 5'-nuclease comprises a thermostable 5'-nuclease.

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~~121.~~ The method of Claim ³¹~~120~~, wherein a portion of the amino acid sequence of said

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nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase from a thermophilic organism.

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122. The method of Claim ³²121, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

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123. The method of Claim ¹⁸107, wherein said detecting the cleavage of said cleavage structure comprises:

- a) providing:
 - i) said non-target cleavage product;
 - ii) a composition comprising two single-stranded nucleic acids annealed so as to define a single-stranded portion of a protein binding region; and
 - iii) a protein; and
- b) contacting said non-target cleavage product and said single-stranded portion of said protein binding region under conditions such that said non-target cleavage product and said single-stranded portion of a protein binding region hybridize to form a double stranded protein binding region, and wherein said protein binds to said double stranded protein binding region.

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124. The method of Claim ³⁴123, wherein said protein comprises a nucleic acid polymerase and wherein said nucleic acid polymerase binds to said protein binding region and produces nucleic acid.

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125. The method of Claim ³⁵124, wherein said protein binding region is a template-dependent RNA polymerase binding region.

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126. The method of Claim ³⁶125, wherein said template-dependent RNA polymerase binding region is a T7 RNA polymerase binding region.

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~~127.~~ The method of Claim ~~107~~¹⁸, wherein said detecting the cleavage of said cleavage structure comprises:

- a) providing:
 - i) said non-target cleavage product;
 - ii) a single continuous strand of nucleic acid comprising a sequence defining a single strand of an RNA polymerase binding region;
 - iii) a template-dependent DNA polymerase; and
 - iv) a template-dependent RNA polymerase;
- b) contacting said non-target cleavage product and said RNA polymerase binding region under conditions such that said non-target cleavage product binds to a portion of said single strand of said RNA polymerase binding region to produce a bound non-target cleavage product;
- c) contacting said bound non-target cleavage product and said template-dependent DNA polymerase under conditions such that a double-stranded RNA polymerase binding region is produced; and
- d) contacting said double-stranded RNA polymerase binding region and said template-dependent RNA polymerase under conditions such that RNA transcripts are produced.

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~~128.~~ The method of Claim ~~127~~³⁸, further comprising the step of e) detecting said RNA transcripts.

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~~129.~~ The method of Claim ~~127~~³⁸, wherein said template-dependent RNA polymerase is T7 RNA polymerase.

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~~130.~~ The method of Claim ~~107~~¹⁸, wherein said target nucleic acid comprises single-stranded DNA.

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~~131.~~ The method of Claim ~~107~~¹⁸, wherein said target nucleic acid comprises double-stranded DNA and prior to step c), said reaction mixture is treated such that said

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double-stranded DNA is rendered substantially single-stranded.

⁴³ 132. The method of Claim ⁴² 131, wherein said double-stranded DNA is rendered substantially single-stranded by heat.

⁴⁴ 133. The method of Claim ¹⁸ 107, wherein said reaction conditions comprise providing a source of divalent cations.

⁴⁵ 134. The method of Claim ⁴⁴ 133, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.

⁴⁶ 135. The method of Claim ¹⁸ 107, wherein said first and said second oligonucleotides are provided in concentration excess compared to said target nucleic acid.

⁴⁷ 136. The method of Claim ¹⁸ 107, further comprising providing a third oligonucleotide complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid, wherein said third oligonucleotide is mixed with said reaction mixture in step b); and wherein said third oligonucleotide is annealed to said third portion of said target nucleic acid.